

Effects of hydrography on the distribution of bacteria and virus in Cochin estuary, India

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Abstract

The viral dynamics have rarely been investigated in estuarine environments of India. The present study brings out a firsthand information on the distribution of virus and bacteria in an eutrophic estuary (Cochin, India). Thirteen stations were selected for monthly monitoring of bacterial abundance (BA), total viable bacterial count (TVC), viral abundance (VA), chlorophyll *a* (Chl *a*) and other water quality parameters (temperature, salinity, pH, dissolved oxygen and inorganic nutrients such as NO₃-N, NH₄-N, PO₄-P and SiO₄-Si) for 1 year. There was significant variations in the VA (0.59 to 4.48 ×10⁷ viral like particles (VLPs) mL⁻¹) and BA (0.49 to 8.12 ×10⁶ cells mL⁻¹) in the estuary. The variation in the viral to bacterial ratio (3 - 22) indicated marked seasonality. Statistical analysis showed bacteria as a major factor controlling the distribution of viruses (60% variability) in Cochin estuary (CE). The viruses also showed positive correlations with Chl *a*, pH and salinity. The distribution of virus followed a distinct pattern of three different zones in the estuary controlled by salinity in zone I, Chl *a* in zone II and salinity, Chl *a* in zone III. The zonal distribution suggests that the factors that control the viral-host systems vary in different areas due to the complex hydrography of the estuary and environmental changes are very sensitive to the viral population in CE.

Key words: Viral abundance; Bacterial abundance; Viral to bacterial ratio; seasonal dynamics; Cochin estuary.

Introduction

Cochin estuary (CE) is one of the largest tropical estuaries (256 km²) along the south west coast of India (Qasim, 2003). The freshwater discharge during the southwest monsoon (MON) has a tremendous impact on this estuary, as it receives 75% of rains (av. ~ 3200 mm yr⁻¹) between June and September (Srinivas, *et al.*, 2003). Based on the propagation of tides and currents, the estuary has been divided into different zones indicating the complex nature of the system (Ramamirtham and Muthusamy, 1986; Balachandran *et al.*, 2008). Importantly, the CE has undergone drastic transformations over the past five decades and the increased microbial activity has transformed it from an autotrophic to a highly heterotrophic system (Thottathil *et al.*, 2008a; Gupta *et al.*, 2009).

The viruses play a crucial role in lysing bacterial and phytoplankton cells to release the dissolved organic matter and thereby increasing the community respiration and nutrient recycling (Middelboe *et al.*, 2001; Winter *et al.*, 2004; Martínez *et al.*, 2007). The viral activity is generally dependent on the quality of water as well as the available hosts (Murray & Jackson, 1992; Clasen *et al.*, 2008). Despite some studies on the bacterial metabolic capabilities (Thottathil *et al.*, 2008a, 2008b), their antibiotic/metal resistance (Parvathi *et al.*, 2009; Parvathi *et al.*, 2011; Jose *et al.*, 2011) and the virioplankton abundance (Parvathi *et al.*, 2013), information on the dynamics and distribution of viruses in CE is largely unknown. Hence studies on the viral dynamics are essential to evaluate their contribution to the heterotrophic behavior of the CE. The present study therefore, aims to investigate the abundance and distribution of bacteria, and virus and their preferences to different zones in the CE.

Methods

Site description and sampling procedure

The Cochin estuary (CE) is one of the productive estuarine systems along the south west coast of India. It is connected to the Arabian Sea through two inlets, at Cochin (width 450 m) and Azhikode (width 250 m) with channel depths of ranging from 5 and 14 m respectively. The estuary is generally wide (0.8–5.5 km) and deep (4–13 m) towards south but becomes narrow (0.3–1.5 km) and shallow (0.5–3.0 m) towards north (Balachandran *et al.*, 2008). The six rivers that discharge freshwater ($2.0 \times 10^{10} \text{ m}^3 \text{ y}^{-1}$) into this estuary are Periyar, Pampa, Achankovil, Manimala, Meenachil and Muvattupuzha (Srinivas *et al.*, 2003; Shivaprasad *et al.*, 2013). The estuary remains fresh water dominated during MON (June–September), but progressively changes to partially mixed during November–January and well-mixed during February–May (Qasim, 2003). During the present study (2008–09), 13 stations were occupied in

the CE at monthly interval for hydrographic sampling (Fig 1). Stations 1 and 6 represent the two inlets at Cochin and Azhikode respectively, whereas stations 2, 3, 4 and 5 are located towards the northern side of the Cochin inlet (St 1) and stations 7 to 13 are located towards the southern side of the Cochin inlet.

Physico-chemical parameters

Temperature and salinity were measured using a conductivity temperature density (SBE Seabird 19 CTD) profiler (accuracy $\pm 0.001^\circ\text{C}$ for temperature and ± 0.001 S/m for conductivity). Salinity was also measured using an Autosal (Guild line) for correcting the CTD salinity. Water samples collected from 0.5 m below the surface using a 5L Niskin sampler (Hydro-Bios, Germany) were kept in ice boxes, brought to the laboratory within 6 h of collection and analyzed for dissolved inorganic nutrients such as nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), ammonia ($\text{NH}_4\text{-N}$), phosphate ($\text{PO}_4\text{-P}$) and silicate ($\text{SiO}_4\text{-Si}$) spectrophotometrically following standard procedures (Grasshoff et al., 1999). Water samples collected for dissolved oxygen (DO) was estimated according to Winkler's titration method.

Biological parameters

For the estimation of microbiological parameters, water samples collected from each station were transferred to 30 ml sterile acid washed polypropylene bottles, kept in iceboxes and brought to the laboratory. The samples were stained with SYBR green I fluorescent dye (Invitrogen, CA, USA) as previously described (Patel et al., 2007). Briefly, 1 ml of the sample was filtered through a 0.02 μm pore-size (25 mm diameter) anodisc filter (Al_2O_3 coated, Whatman) backed by 0.8 μm cellulose mixed ester membrane (Millipore) under vacuum (20 Kpa). A 2.5 μl of SYBR green I (diluted 1:10) was mixed with 97.5 μl of sterile viral-free water on the bottom of a sterile plastic petridish to get a final concentration of 1:400. The anodisc filter was stained in this solution and incubated for 15 min in the dark. The filter was then transferred to a clean glass slide and kept over a cover slip containing 30 μl antifading solution [50% glycerol, 50% PBS - phosphate buffered saline (0.05M Na_2HPO_4 , 0.85% NaCl, pH 7.5), 0.1% p-phenylene diamine]. The slides were either immediately counted or stored at -20°C until enumeration. For each filter, 10-20 fields were selected randomly and a total of >200 viruses and >200 bacteria were counted at $1000\times$ using an epifluorescence microscope (Olympus BX 41) under blue excitation. Total viable counts (TVC) were measured to estimate the physiologically active bacteria as previously described (Hobbie et al., 1977). Briefly, 5 ml water sample was mixed with 50 μl of 0.05% yeast extract, 50 μl of antibiotic cocktail (nalidixic acid, pipemidic acid and piromidic acid) and incubated in dark for 6 hours. The samples were fixed in 2% formalin and the swollen (elongated) cells

were stained with 100 µl of acridine orange (0.1g/100 ml) and enumerated using epifluorescence microscope.

Chlorophyll *a* (Chl *a*) and pheophytin (acidified with 0.1 N HCl) concentrations were determined by passing water samples (500 ml) through 0.7 µm, 47 mm dia GF/F filters (Whatman, USA). The pigments retained on the filters were extracted with 90 % acetone for 24 h in the dark at 4°C (Parsons et al. 1984) and the fluorescence was measured using a fluorometer (Model 7200-000, Turner Designs, Trilogy, USA). The measurement of pheophytin is quite relevant in aquatic systems exhibiting high organic production such as CE (Qasim, 2003). Pheophytin represents the dead or degraded Chl *a* and can normally range up to 30% of total phytoplankton biomass.

Statistical analysis

The biological components such as bacterial abundance (BA), viral abundance (VA) and total viable count (TVC) were subjected to Karl Pearsons' correlation analysis to identify their preference to water quality parameters. A step up multiple regression (SMR) analysis after performing log transformation and normalization of all variables (Snedecor and Cochran, 1967; Jayalakshmy, 1998) was used to explain their inter-relationship. Out of the 16 models fitted, the one with the maximum explained variability was selected to explain their interactions.

Further, a three way ANOVA was performed to characterize the interaction of virus with various environmental parameters (temperature, salinity, DO, inorganic nutrients) and biological factors (BA, VA, TVC, Chl *a*, pheophytin) in the different estuarine locations with respect to time. Based on the 3 way ANOVA, the observation period (12 months) was grouped into three seasons such as pre monsoon (PRM), post monsoon (PM) and south west monsoon (MON). A canonical discriminant analysis (CDA) was used to identify estuarine locations with similar characteristics. A partial correlation analysis was performed to determine the factors influencing the distribution of virus in the CE.

Results

Spatial and temporal variations in physico-chemical parameters

During the study period, the air temperature ranged from 26° C to 34.4° C with a maximum during March (PRM) and minimum during December (PM). The median values of various physico-chemical and biological parameters with upper and lower quartile values are shown in Figure 2. The mean water temperature ranged from 25.5 to 33.2°C with St 7 and 4 showing the maximum and the minimum

temperatures respectively. The seasonal fluctuations in salinity was maximum during PRM (20.06 ± 6.96) followed by PM (15.09 ± 9.23) and MON (5.45 ± 4.51). The measured hydrographic parameters were generally different for the north and south estuaries. The increase in the fresh water discharge during monsoon has considerably reduced the salinity (Fig 2 and S1), as the estuary was predominantly fresh water dominated, except at the inlets (salinity 2 at St 1, 6). Station 1, 2, 7, 8 and 9 represent the central estuary, stations 3,4 and 5 represent the north estuary and stations 10-13 represent the south estuary. The south estuary (St 11, 12, and 13) remained fresh water dominant from June to early December, but reached maximum during the PRM (Fig 2). Salinity was relatively high (av. 25.20 ± 6.43) in the north estuary during PRM compared to the south (av. 15.65 ± 3.65) estuary (Fig S2). The trend was almost similar during PM (Fig 2, S2), as the north estuary (18.92 ± 8.11) continued to be high saline than the south estuary (av. 11.82 ± 9.41).

The variations in the biological parameters and salinity during the three seasons are shown in Figure S1, S2). The estuary remained saturated with respect to DO, which was maximum during the PM and minimum during MON (Fig 2). Similarly, pH was maximum during the PM and minimum during MON (Fig 2). NH_4 ($0.2\text{-}95.3 \mu\text{M}$) concentrations were lowest during PRM, whereas SiO_4 was maximum during MON and minimum during PRM (Fig 2).

Seasonal and spatial variations in VA, BA, TVC and chlorophyll a

The abundances of bacteria and virus exhibited distinct spatial and seasonal patterns (Fig S1). The BA ranged from 0.49×10^6 to 8.1×10^6 cells mL^{-1} with lowest and highest values at Stn 6 and Stn 5 respectively (Fig 2). In the case of TVC, the values ranged from 0.11×10^6 to 1.37×10^6 cells mL^{-1} with lowest value at Stn 8 and the highest at Stn 3. The VA ranged from 5.92×10^6 to 44.86×10^6 VLPs mL^{-1} with lowest value at Stn 10 and highest at Stn 12. The lowest and highest values of BA, TVC and VA were during MON and PRM respectively. The virus to bacteria abundance ratio (VBR) was used to examine the relationship between the abundance of viral and bacterial populations. The VBR varying from 3 to 22 in this study, is almost consistent in nutrient rich waters suggesting a tight coupling between bacterial and viral concentrations and relatively constant levels of virus production and loss.

There was marked spatial and temporal variations in the Chl *a* ($0.33\text{-}90.1 \text{ mg m}^{-3}$) biomass during the study period (Fig 2). The Chl *a* was maximum during PRM with an exceptionally high value of 90 mg m^{-3} at station 3, where the pheophytin (35 mg m^{-3}) concentration was also high (ESM, Fig S2). The chlorophyll: pheophytin ratio showed marked seasonal variation from 1.73 during MON to 30.19 during

PM in zone 1. The south estuary (Stns 9-13) sustained relatively low Chl *a* during all the seasons, whereas Stn 6 and Stn 7 showed the least temporal variations. MON period was characterized by a relatively low Chl *a* concentrations when compared to other 2 seasons (ESM, Fig S2).

Statistical analysis

Correlation analysis showed BA as the best predictor of VA in CE irrespective of space and time (Fig 3, Table 1). The VA showed significant positive correlations ($p = 0.001$) with BA ($r = 0.71$, $n=156$), salinity ($r = 0.35$, $n = 156$), TVC ($r = 0.20$, $n = 156$) and Chl *a* ($r = 0.20$, $n=156$) (Fig 3). VBR showed a clear association with BA and was significantly negatively correlated with BA ($r = -0.56$, $n = 156$). The 3-way ANOVA delineated significant differences in the BA and VA between stations and months (Table S1). A canonical discriminant analysis (CDA) with jackknife cross validation technique was performed for each season to determine stations with similar characteristics, their shifts and discriminative parameters. The CDA revealed that physico-chemical parameters except temperature and NO_3 are important discriminating parameters of viruses and bacteria (Variability explained, $\text{VE} = 71\%$) in the CE. Thus the viral distribution in the CE can be pooled into 3 zones depending on the hydrological behavior (Fig 4 and 5). These zones are; zone 1, consisting of stations *viz*: 1, 2, 6, 7, 8 and 9, zone 2 with stations 3, 4 and 5 and zone 3 consisting of stations from 10 to 13. This is further supported by the SMR results to show that in addition to BA, the major factors influencing the VA is salinity in zone 1 ($\text{VE} = 74.87\%$); Chl *a* in zone 2 ($\text{VE} = 94.29\%$), and salinity and Chl *a* in zone 3 ($\text{VE} = 85.79\%$) respectively (Table 1).

Zonal variations in viral distribution

The distribution of viruses in different zones of the estuary is evident from Fig 4 and 5. The distribution was generally in response to the changes in the salinity and biological parameters (BA, TVC, Chl *a* and pheophytin). The zonal characteristics are even more evident during the PM and PRM when the salinity progressively increases in the estuary. Thus, the average VA in zone 1 was $24.21 \pm 3.36 \times 10^6$ VLPs mL^{-1} , when compared to $10.46 \pm 3.36 \times 10^6$ VLPs mL^{-1} in zone 3 during PRM. The abundance of viable bacteria (TVC) was found to significantly influence of the zonal distribution of VA ($r=0.94$, $p<0.05$), followed by salinity ($r=0.92$, $p<0.05$) (Fig 5). Zone 2 showed greater seasonal variations in Chl *a* and pheophytin when compared to the other zones.

Discussion

The main aim of this study was to make a first-time estimate of the viral and bacterial population and to examine their pattern of distribution in the CE. Although their distribution followed a similar pattern, bacteria and phytoplankton are considered as the major hosts for viruses (Mann, 2003). Since bacteria are more abundant than phytoplankton, viruses prefer bacteria (Jacquet *et al.*, 2010) and multiply faster in areas where the bacteria are high. VA showed significant correlation with TVC, indicating their preference to live and physiologically active bacteria for their attachment and infection. The results showing a positive correlation for VA with salinity and negative correlations with inorganic nutrients such as NO₃, PO₄ and SiO₄ indicate their sensitivity to salinity and nutrients (Wilhelm and Suttle, 1999; Wommack and Colwell, 2000). Studies in the Delaware Bay (Cottrell and Kirchman, 2003), Chesapeake Bay (Bouvier and del Giorgio, 2002) and South China Sea (Zhang *et al.*, 2006) have also reported bacterial community shift over a salinity gradient. However, absence of correlation for BA and VA with temperature could indicate the strong adaptation of bacteria and virus with tropical climate. This is not surprising because a temperature variation of ~ 26 - 34° C in tropical environment could favor an optimal virus growth. The significant positive correlation between VA and Chl *a* possibly indicates that viruses are partly responsible for the mortality of phytoplankton cells. The significant correlations obtained for VA with Chl *a* and BA are consistent with the results obtained for eutrophic estuaries (Jiang and Paul, 1994).

VBR is influenced by several factors that control their production and loss (Wommack and Colwell, 2000). The variation in the VBR (3 – 22) could be due to the dynamic nature of virus. VBR generally remained high in nutrient-rich location, indicating that higher bacterioplankton favour increased virus production. Virus production can also increase due to higher infection rate and larger burst size (Wommack and Colwell, 2000). The VBR in this study was comparable with those reported for Chesapeake Bay (12.6 to 25.6) (Wommack *et al.*, 1992) but higher than that in the Tampa Bay (0.9 to 9.1) (Jiang & Paul, 1994). The virus population in CE are largely sensitive to bacterial population rather than phytoplankton biomass in CE. However, a high VBR with a low BA were recorded and vice versa, possibly caused by increased cell abundance resulting in more infections, or a lower residence time of viruses (Jiang & Paul 1994).

Viruses and bacteria showed distinct pattern of distribution in different zones of the estuary, following the changes in the physic-chemical characteristics (Fig.4, 5). Microbes are known to follow distinct

biogeographic patterns, but how they maintain the heterogeneity is still unknown (Hughes Martiny et al., 2006). It is widely accepted that viruses prefer regions where the bacteria and phytoplankton are high (Cochlan et al., 1993). The SMR and CDA results from the present study have suggested that the virus distribution in the CE follow distinct zonal patterns. It is therefore likely that these different zones identified based on the biochemical properties exhibit unique hydrodynamic features. This is because CE has been classified into 3 zones, where the northern and southern parts of the estuary is embedded into a centrally located dynamic zone (Balachandran et al., 2008). They have attributed that the unique topography and tidal asymmetry are responsible for the development of zones with variable flow and flushing characteristics. Our results corroborate the above concept to show that differences in properties of these zones are responsible for the observed difference in the viral distribution. It has been shown that the prokaryotes and viruses, unlike the macroscopic organisms are passive in dynamic water bodies (Snyder et al., 2007; Cho and Hwang, 2011). The physic-chemical properties of these zones have significantly influenced the virus distribution. The Zone 1 representing the central estuary, is dynamic and hence mainly influenced by tides and salinity. The zone 2 representing the north estuary has a relatively high residence time. This zone is also a recipient of large quantities of effluents from industries. The zone 3 representing the south estuary is weak in its water renewal. This zone is a recipient of seepage water from upland agricultural fields.

In zone 1, BA and salinity are the major factors controlling the VA. Thus is because salinity is a major factor supporting halotolerant bacteria in CE (Thottathil et al., 2008b). The study reported that during high saline period, proliferation of autochthonous and halotolerant bacterial community occurs with increased utilization of carboxylic/amino acids, the major components of DOM in this estuarine environment. Thus, the high tidal activity and salinity in the lower estuary (zone 1) was favorable for an increased microbial activity. As a consequence, the VA in this region was also found to be different from the other two zones.

In the zone 2 (north estuary), the virus distribution is influenced by Chl *a* in addition to BA. This zone 2 (Stns 3-5) is relatively stagnant, where effluents from industries get accumulated leading to enhanced phytoplankton production. During PRM, the phytoplankton species composed of *Cylindrotheca closterium* (2.1×10^5 cells L⁻¹) and *Nitzschia longissima* (2.0×10^4 cells L⁻¹) were maximum in this zone (4.4×10^5 cells L⁻¹, Chl *a*, 90 mg m⁻³) implying that high nutrient concentrations have caused enhanced phytoplankton growth. The region receives a lot of nutrients industrial run off and terrestrial wastes which is not flushed out of the system as in the zones. Hence nutrients may support a stable

phytoplankton population. During such instances of high phytoplankton biomass, the virus may prefer phytoplankton as host due to increased surface area (Cochlan, 1993). The pheophytin concentration in the CE was also high (35 mg m^{-3}) in this zone during PRM, suggesting that mortality of phytoplankton due to viral lysis is significant. More studies are required to corroborate this as zooplankton grazing could also increase the pheophytin levels. The above observations show that host availability is a critical factor deciding virus community composition (Winter *et al.*, 2010).

In zone 3, BA, salinity and Chl *a* are the influencing factors of VA. This zone also experiences sluggish water movement and tidal activity. Due to wide storage area in the zone 3, the tides are considerably reduced in this region leading to consistently low salinity and Chl *a* (Balachandran *et al.*, 2008). This region is also characterized by the presence of autochthonous limnotolerant bacteria (Thottathil *et al.*, 2008 b), which do not tolerate fluctuations in salinity. Thus, the VA remained at its minimum in this zone when compared to the other zones.

In the present study, we observed that even a small ecosystem such as CE sustains a very high population of bacteria and virus. Gradients in the environmental properties are found to influence the viral distribution in oceanic and polar waters and larger lakes (Angly *et al.*, 2006; Van der Gucht *et al.*, 2007; Held and Whitaker, *et al.*, 2009; Winter *et al.*, 2013). To sum up, the virus distribution in the CE is influenced by factors such as BA, Chl *a* and salinity, whereas they show distinct pattern of distribution in different parts of the estuary. The flow restrictions of these zones, together with increased human activities have resulted in a rich viral population in CE, which was in accordance with the zonal classification of this estuary. It also indicates that factors that control the viral-host systems vary in different areas within the estuary. This information will be useful to evaluate the effects of environmental changes on bacteria and virus and for the environmental management of CE. Since virus plays a significant role in sustaining diverse planktonic communities, future studies should include viruses as an important component in the productivity cycles to quantify carbon flux in eutrophic ecosystems.

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Figure Legends:

Fig 1: Station locations in the Cochin estuary (CE). Stations 1 and 6 are the two inlets. Station 1, 2, 7, 8 and 9 represent the central estuary, stations 3, 4 and 5 represent the north estuary and stations 10-13 represent the south estuary. The boxes representing the different zones (1, 2 and 3) in the estuary are demarcated based on the results of canonical discriminant analysis (CDA).

Fig 2: Boxplots representing the variations in the physico-chemical and biological parameters in Cochin estuary (CE). The median, upper and lower quartile values of various physico-chemical and biological parameters are represented in this figure. The plus and minus error bars of standard deviation is indicated in the figure. The panel (a) represents viral abundance (VA) in viral like particles (VLPs) $\times 10^6$ mL⁻¹, (b): bacterial abundance (BA) in $\times 10^6$ mL⁻¹, (c): total viable bacteria (TVC) in $\times 10^6$ mL⁻¹, (d): Chlorophyll *a* (Chl *a*) in mg m⁻³, (e): salinity as practical salinity unit (psu), (f): temperature in °C, (g): dissolved oxygen (DO) in mg L⁻¹, (h): inorganic nutrients NH₄-N in μ M, (i): pH and (j): SiO₄-Si in μ M

Fig 3. Scatter plots indicating relationships of viral abundance (VA) with bacterial abundance (BA), total viable bacterial count (TVC), chlorophyll *a* and salinity. Panel (a) represents VA \times BA, (b): VA \times TVC, (c): VA \times Chl *a* and (d): VA \times Salinity. VA is represented as viral like particles (VLPs) and salinity as practical salinity unit (psu). The *r*- values are represented on the top right end of each panel, *r* values > 0.2 is significant at $p=0.005$, $n=156$.

Fig 4. Zonal and seasonal characteristics of various parameters (mean \pm SD) during monsoon (MON), post monsoon (PM) and pre monsoon (PRM). Panel (a) represents viral abundance as viral like particles (VLPs), (b): bacterial abundance, (c): viable bacteria, (d): salinity as practical salinity unit (psu), (e): chlorophyll *a* and (f): pheophytin in the Cochin estuary (CE).

Fig 5. Canonical discriminant analysis (CDA) analysis discriminating the three different zones in Cochin estuary (CE). The stations are grouped into three different zones. Different circles indicate different zones that include stations with similar characteristics. Zone 1 includes stations 1, 2, 6, 7, 8, 9, Zone 2 includes stations 3, 4, 5 and Zone 3 includes stations 10, 11, 12, 13.

Table Legends:

Table S1: Table showing results of 3-way ANOVA for comparison of stations and months using Snedecor's *F* statistics. *Indicates cases which are not significant at 5% level of significance. Abbreviations used: BA: Bacterial abundance, VA: Viral abundance, TVC: total viable bacterial count, Chl: chlorophyll *a*, T: water temperature, inorganic nutrients such as NO₃-N, NH₄-N, PO₄-P and SiO₄-Si, Sal: Salinity, DO: Dissolved oxygen.

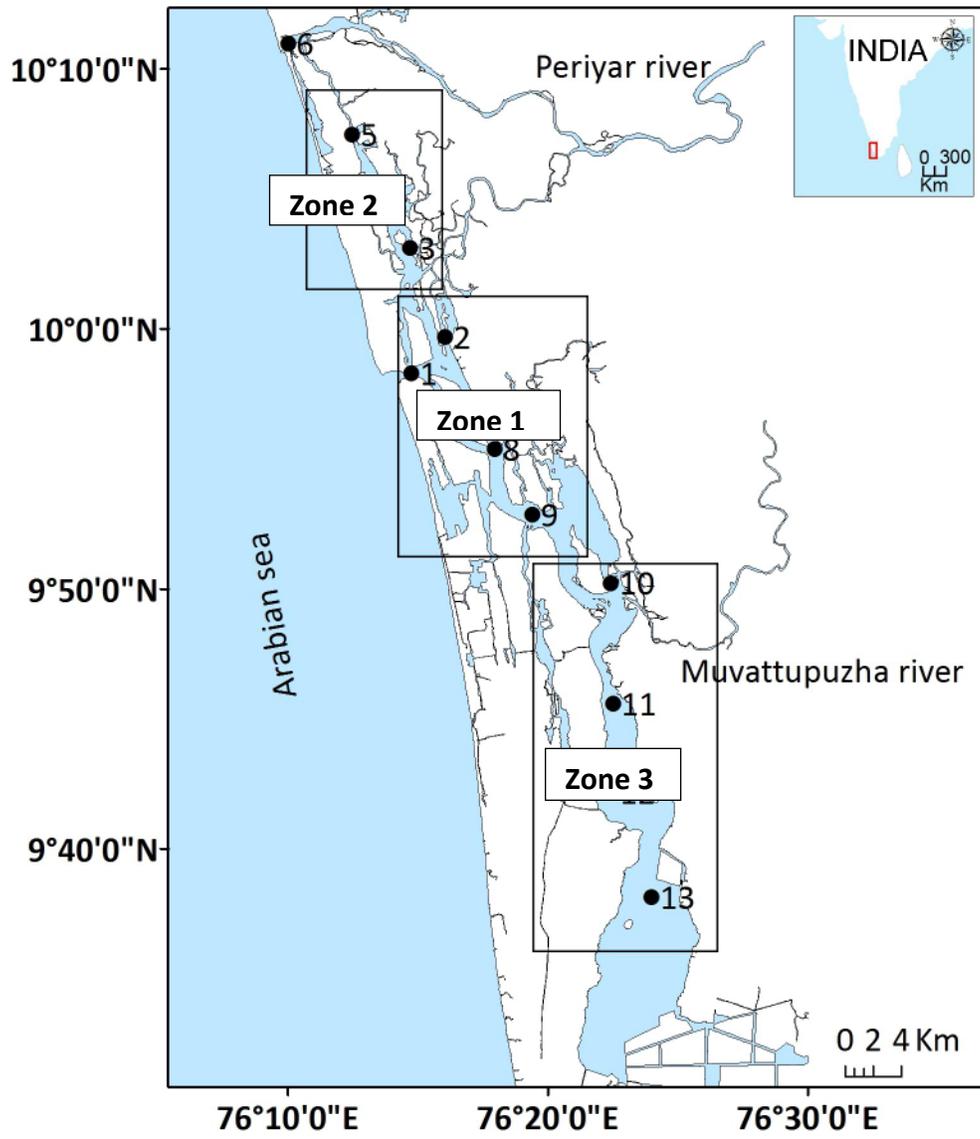


Fig 1

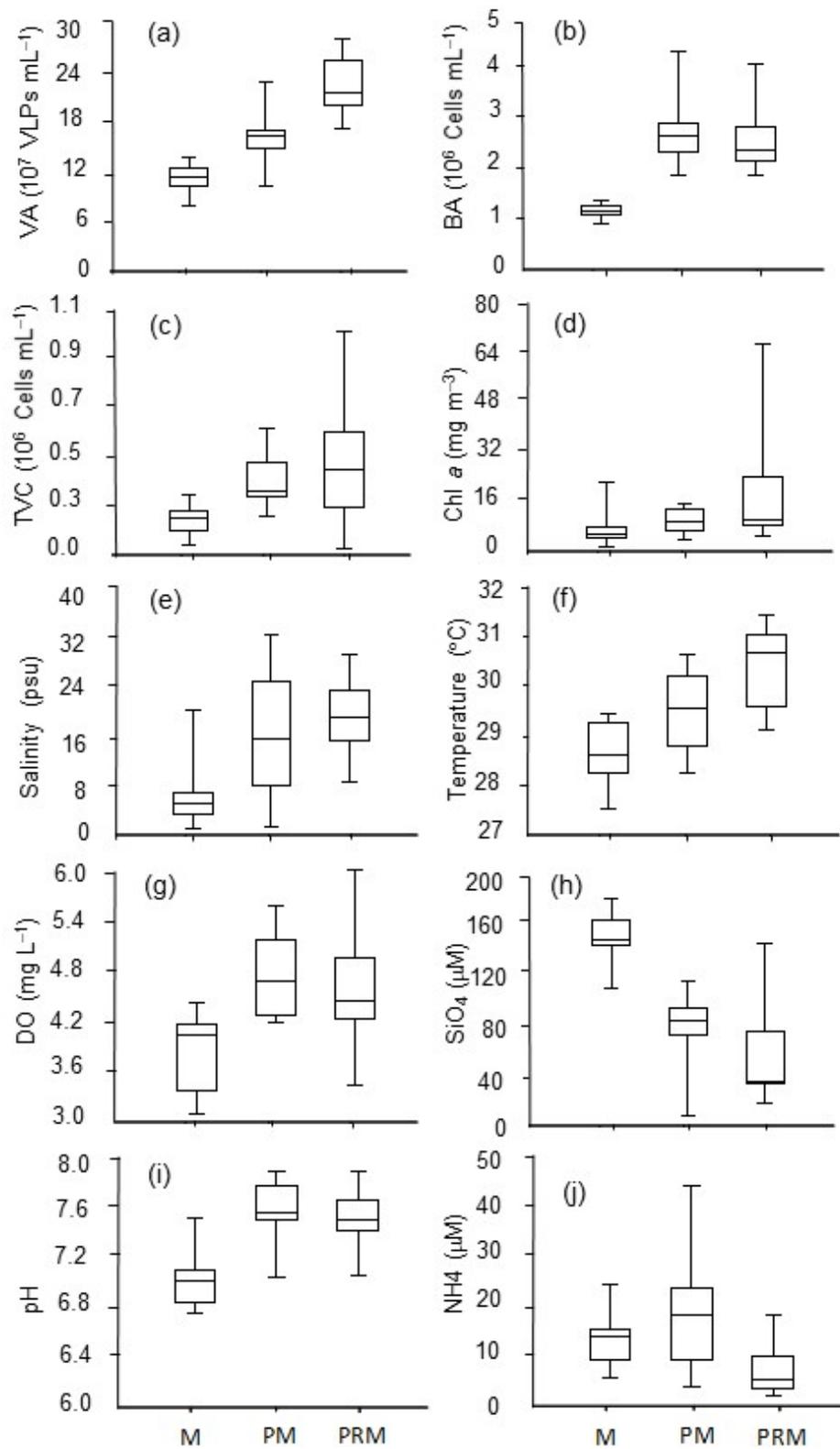


Fig 2

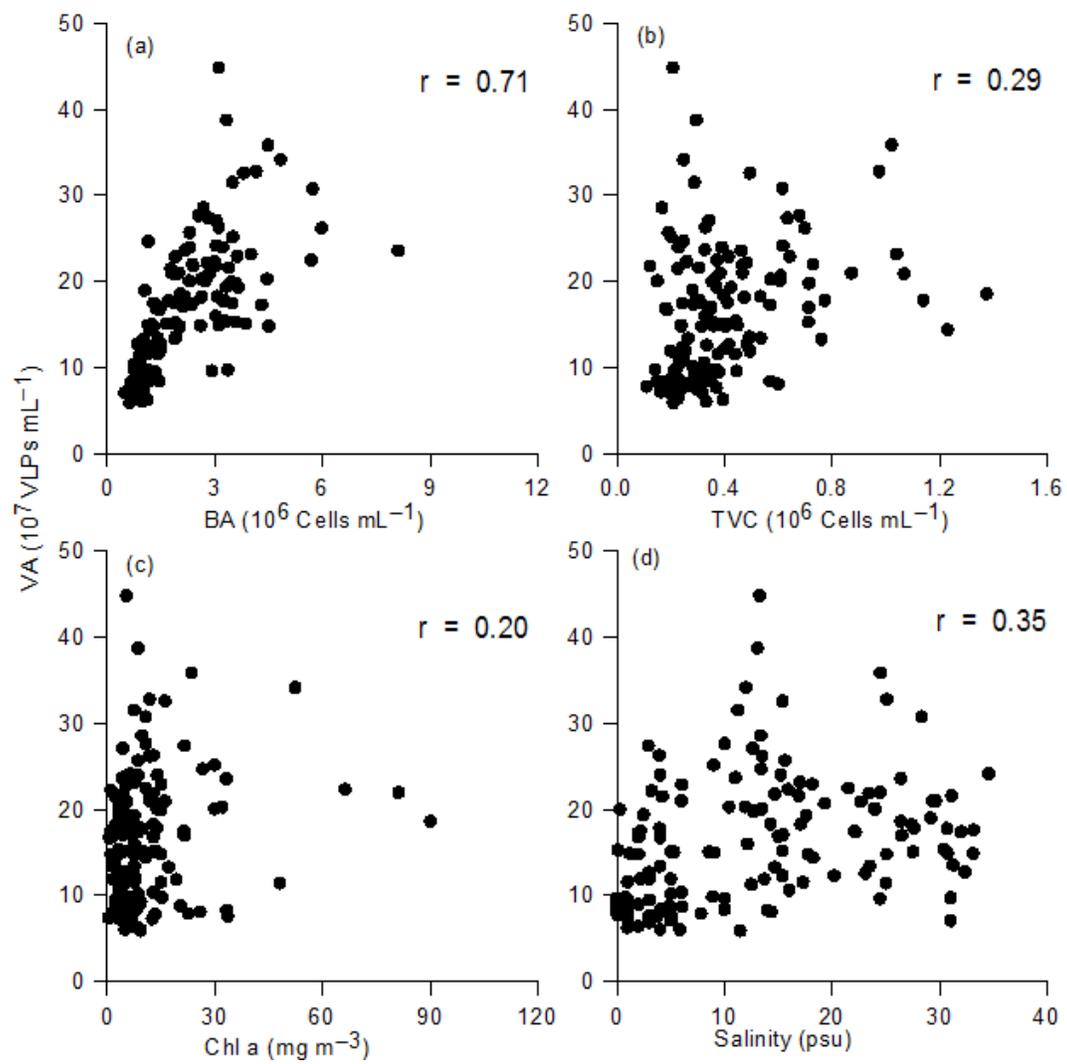


Fig 3

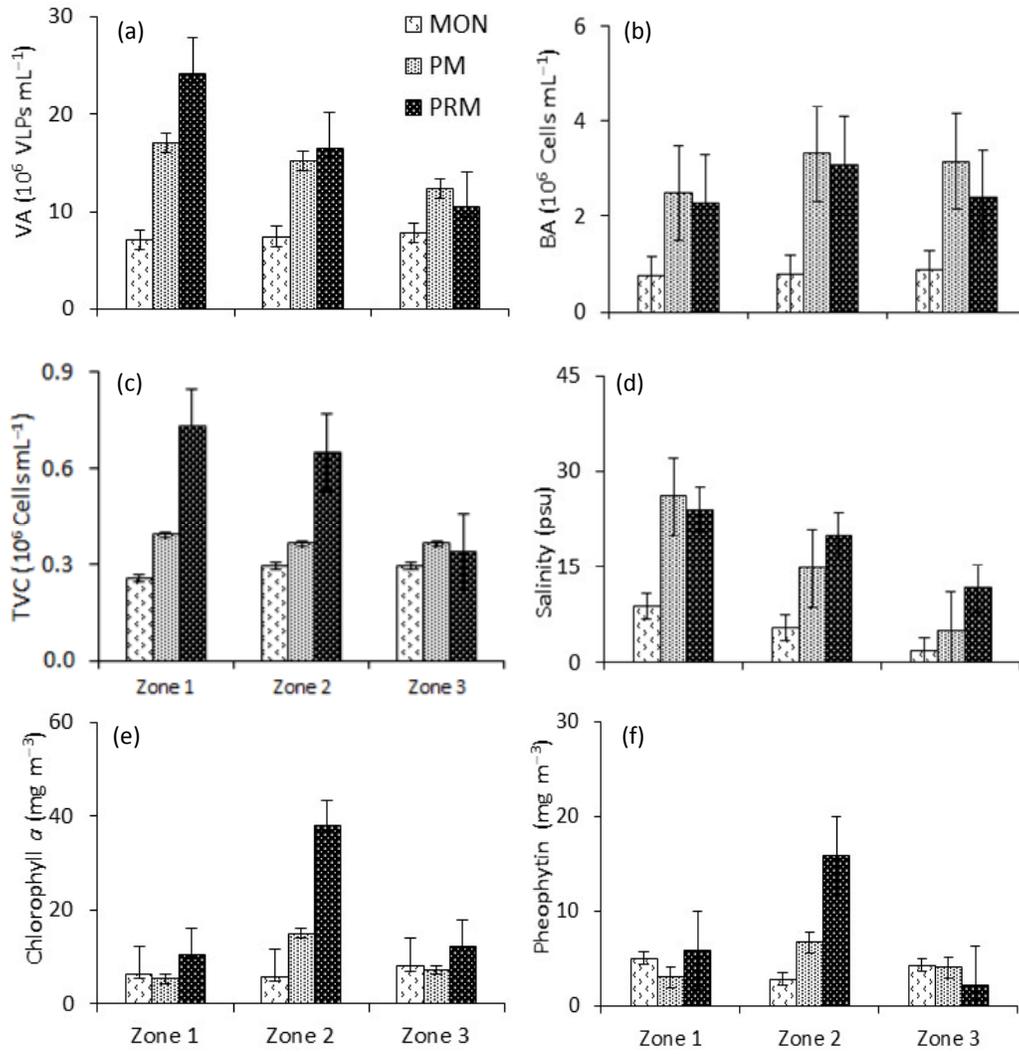


Fig 4

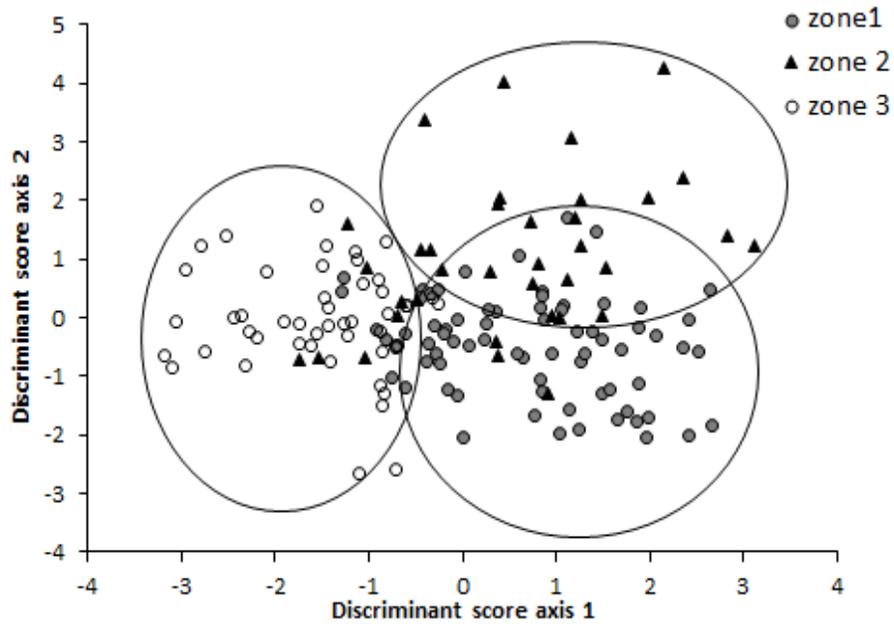


Fig 5

Table 1

Station no.	Independent variables	Significant model	F ratio	DOF	Variability explained (%)
1	BA, WT, NO ₂ , SiO ₄	* BA, NO ₂ , SiO ₄	9.29	(6,5)	81.90
2	BA, WT, DO, SiO ₄	* BA, WT, SiO ₄	15.46	(6,5)	88.75
3	BA, WT, NO ₂ , Chl	* BA, NO ₂ , Chl	11.74	(3,8)	85.43
4	BA, Sal, PO ₄ , Chl	* BA, PO ₄ , Chl	16.46	(6,5)	89.40
5	BA, pH, DO, NH ₄	* BA, pH	15.53	(3,8)	79.85
6	BA, WT, Sal, SiO ₄	* BA, Sal, SiO ₄	10.97	(6,5)	84.47
7	BA, NO ₂ , NH ₄ , Chl	* BA, NO ₂ , Chl	21.16	(6,5)	91.67
8	DO, NO ₂ , NH ₄ , SiO ₄	* NO ₂ , SiO ₄	31.23	(3,8)	89.18
9	BA, pH, DO, PO ₄	* BA, pH, DO	38.48	(6,5)	95.34
10	BA, Sal, NH ₄ , Chl	* BA, NH ₄ , Chl	32.13	(6,5)	94.43
11	BA, WT, pH, Sal	* BA, pH, Sal	9.20	(6,5)	81.74
12	BA, NO ₂ , NH ₄ , SiO ₄	BA, NO ₂ , NH ₄ , SiO ₄	28.62	(4,7)	90.26
13	BA, Sal, NO ₃ , Chl	BA, Sal, NO ₃ , Chl	47.83	(4,7)	94.45

Fig S1

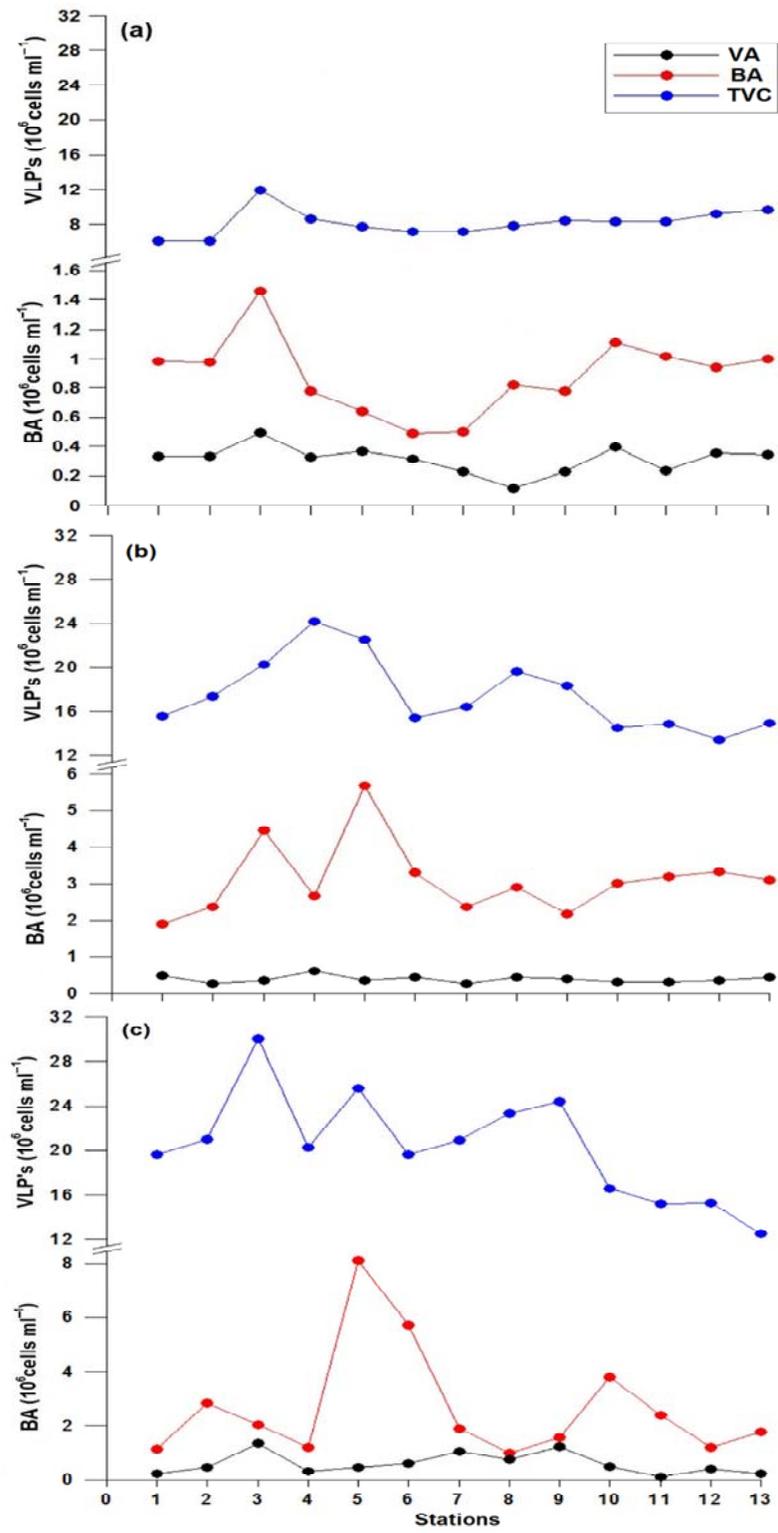


Fig S2

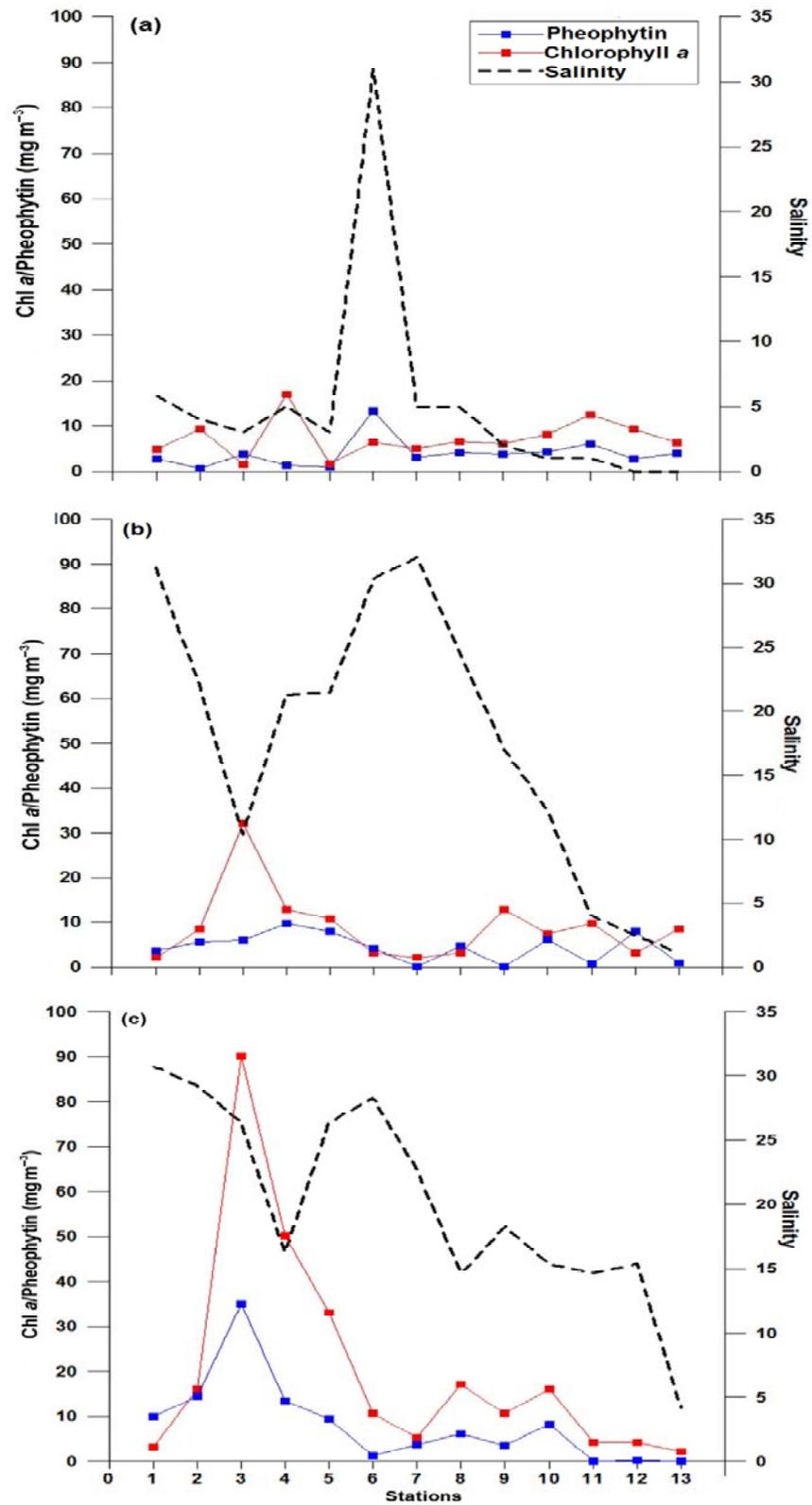


Table S1.

Snedecor's F ratio													
	TVC	BA	VA	T	Chl <i>a</i>	pH	Sal	DO	NO ₂	NO ₃	NH ₄	PO ₄	SiO ₄
Between stations	3.44	7.96	4.79	9.59	4.89	19.61	13.73	3.22	10.51	5.36	2.18	6.17	11.83
Between months	18.36	66.81	44.7	85.29	9.15	151.2	74.28	8.56	65.18	65.68	9.14	30.46	92.66
Stations × months	2.06	3.19	1.65*	2.45	3.98	3.48	3.19	2.53	5.98	0.76*	1.58	3.41	4.04